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Ryncarz
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WHAT IS CLAIMED IS:

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1. In a method for forming multiple copies of a target sequence of a target polynucleotide, said method comprising the step of forming extension products of an oligonucleotide primer at least along said target sequence or along an extended oligonucleotide primer, said extension products being copies of said target sequence, the improvement which comprises forming said extension products in the presence of a second polynucleotide, to which said oligonucleotide primer hybridizes except for the 3'-end of said oligonucleotide primer, under conditions wherein the extension of said oligonucleotide primer along said second polynucleotide is controlled relative to the extension of said oligonucleotide primer along said target sequence.

2. In a method for amplifying a target sequence of a target polynucleotide, said method comprising combining a sample suspected of containing said target polynucleotide with reagents for amplifying said target sequence if present and subjecting said combination to conditions wherein said target sequence if present is amplified, said reagents comprising an oligonucleotide primer and a polymerase, the improvement which comprises (a) including in said combination a

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control polynucleotide, to which said oligonucleotide primer hybridizes except for 1-10 nucleotides at the 3'-end of said oligonucleotide primer, and a 3' to 5' exonuclease when said polymerase does not comprise a 3' to 5' exonuclease, wherein said oligonucleotide primer extends along said target sequence and extends along said control polynucleotide to produce copies of said control polynucleotide only after said 1 to 10 nucleotides are degraded by said polymerase having 3' to 5' exonuclease activity and (b) detecting the presence of said copies of said control polynucleotide, the presence thereof indicating that said reagents and conditions for amplifying said target sequence are functional.

3. The method of Claim 2 wherein said oligonucleotide primer is fully complementary to that portion of said target sequence to which it hybridizes and is complementary to that portion of said control polynucleotide to which it hybridizes except for said 1 to 10 nucleotides at the 3'-end thereof.

4. The method of Claim 2 wherein a modified oligonucleotide primer is included in said combination wherein said modified oligonucleotide primer is substantially identical to said oligonucleotide

primer but contains a chemical modification at its 3'-end that prevents degradation, by said 3' to 5' exonuclease, of said 1 to 10 nucleotides.

5. The method of Claim 4 wherein said chemical modification is selected from the group consisting of phosphorothioates, ethyl phosphonates, carboxamides, sulfonamides, carbamates, acetals and ketals.

6. The method of Claim 4 wherein said chemical modification is a phosphorothioate.

7. The method of Claim 2 wherein said oligonucleotide primer hybridizes to said control polynucleotide except for 3-5 nucleotides at the 3'-end of said oligonucleotide primer.

8. The method of Claim 2 wherein the presence of amplified target sequence is detected and related to the presence of said target polynucleotide.

9. In a method for forming multiple copies of a target sequence of a single stranded target polynucleotide ("target sequence"), said method comprising:

(a) hybridizing to the 3'-end of said target sequence a first oligonucleotide primer ("first primer"),

(b) extending, in the presence of a polymerase, said first primer along at least said target sequence, said first primer being capable of hybridizing to, and being extended along, (1) said extended first primer or (2) an extended second oligonucleotide primer ("second primer") wherein said extended second primer results from the extension of a second primer capable of hybridizing to and extending along a polynucleotide that is complementary (complementary polynucleotide) to said target sequence,

(c) dissociating said extended first primer from said target sequence,

(d) hybridizing, to the 3'-end of said extended first primer, said first or said second primer,

(e) extending said first or said second primer along said extended first primer,

(f) dissociating said extended first primer or said extended second primer from said extended first primer,

(g) hybridizing, to the 3'-end of said extended first or second primer, said first primer, and

(h) repeating steps (e)-(g),

the improvement comprising (i) including, as a positive internal control in the same reaction mixture subjected to steps (a) - (g) above, a control polynucleotide, to which said first or said second primer hybridizes except for 1-10 nucleotides at the 3'-end of said first or said second primer, and a 3' to 5' exonuclease when said polymerase does not comprise a 3' to 5' exonuclease, wherein said first or said second primer extends along said control polynucleotide to produce copies of said control polynucleotide only after said 1-10 nucleotides are degraded by said polymerase having 3' to 5' exonuclease activity and (ii) detecting said copies of said control polynucleotide.

10. The method of Claim 9 wherein said first primer is fully complementary to that portion of said target sequence to which it hybridizes and is complementary to that portion of said control polynucleotide to which it hybridizes except for said 1 to 10 nucleotides at the 3'-end thereof.

11. The method of Claim 9 wherein a modified oligonucleotide primer is included in said combination wherein said modified oligonucleotide primer is substantially identical to said first or said second primer except for a chemical modification at its 3'-end that prevents degradation, by said 3' to 5' exonuclease, of said 1 to 10 nucleotides.

12. The method of Claim 11 wherein said chemical modification is selected from the group consisting of phosphorothioates, ethyl phosphonates, carboxamides, sulfonamides, carbamates, acetals and ketals.

13. The method of Claim 11 wherein said chemical modification is a phosphorothioate.

14. The method of Claim 9 wherein said first or said second primer hybridizes to said control polynucleotide except for 3-5 nucleotides at the 3'-end of said first or said second primer.

15. The method of Claim 9 wherein the presence of said extended first primer and/or said extended second primer is detected and related to the presence of said target polynucleotide.

16. The method of Claim 9 wherein said control polynucleotide is 50 to 5000 nucleotides in length.

17. The method of Claim 9 wherein said control polynucleotide is present in the reaction mixture at a concentration of from about 1pM to 100pM.

18. The method of Claim 9 wherein the repeating of steps (e)-(g) is achieved by repeated temperature cycling.

19. The method of Claim 18 wherein temperature cycling is repeated at least 3 times.

20. The method of Claim 9 wherein said target polynucleotide is DNA.

21. The method of Claim 9 wherein said extending is carried out in the

presence of nucleoside triphosphates.

22. The method of Claim 9 wherein said control polynucleotide contains at least a 15 nucleotide sequence that is not in the target sequence.

23. The method of Claim 9 wherein said first and said second primers are different and said control polynucleotide contains a sequence at its 5'-end that is identical to the sequence at the 5'-end of said second primer.

24. The method of Claim 9 wherein said first and said second primers are different and said extended first primer is a template for said second primer and said extended second primer is a template for said first primer.

25. A method for forming multiple copies of at least one double stranded polynucleotide ("polynucleotide"), said polynucleotide comprising a single stranded target polynucleotide sequence ("target

sequence") and its complementary sequence (complementary sequence), said method having a positive internal control, said method comprising:

(a) treating a sample suspected of containing one or more of said double stranded polynucleotides with (i) oligonucleotide primers capable of hybridizing to a portion of each target sequence and its complementary sequence suspected of being present in said sample under conditions for hybridizing said primers to and extending said primers along said target sequence and said complementary sequences, wherein said primers are selected such that the extension product formed from one primer, when it is dissociated from its complement, can serve as a template for the formation of the extension product of another primer, (ii) a control polynucleotide, as a template to which one of said primers hybridizes except for 1-10 nucleotides at the 3'-end of said one of said primers, and (iii) a 3' to 5' exonuclease wherein said primers extend, respectively, along said target sequence and said complementary sequence and said one of said primers extends along said control polynucleotide only after said 1-10 nucleotides are degraded by said 3' to 5' exonuclease,

(b) dissociating primer extension products from their respective templates to produce single stranded molecules and

(c) treating the single stranded molecules produced in step (b) with the primers of step (a) under conditions such that a primer extension product is formed using the single strands produced in step (b) as templates, resulting in amplification of the target sequences and complementary sequences if present, said conditions allowing for the extension of said one of said primers along said control polynucleotide to provide said positive internal control.

26. The method of Claim 25 wherein said one of said primers is fully complementary to that portion of said target sequence to which it hybridizes and is complementary to that portion of said control polynucleotide to which it hybridizes except for said 1 to 10 nucleotides at the 3'-end thereof.

27. The method of Claim 25 wherein a modified oligonucleotide primer is included in said combination wherein said modified oligonucleotide primer is substantially identical to said one of said primers except for a chemical modification at its 3'-end that prevents degradation, by said 3' to 5' exonuclease, of said 1 to 10 nucleotides.

28. The method of Claim 27 wherein said chemical modification is selected from the group consisting of phosphorothioates, ethyl phosphonates, carboxamides, sulfonamides, carbamates, acetals and ketals.

29. The method of Claim 27 wherein said chemical modification is a phosphorothioate.

30. The method of Claim 25 wherein said one of said primers hybridizes to said control polynucleotide except for 3-5 nucleotides at the 3'-end thereof.

31. The method of Claim 25 wherein the presence of primer extension products is detected and related to the presence of said target polynucleotide.

32. The method of Claim 25 wherein said control polynucleotide is 50 to 5000 nucleotides in length.

33. The method of Claim 25 wherein said control polynucleotide is present in the reaction mixture at a concentration of from about 1pM to 100pM.

34. The method of Claim 25 wherein the repeating of steps (a)-(c) is achieved by repeated temperature cycling.

35. The method of Claim 34 wherein temperature cycling is repeated at least 3 times.

36. The method of Claim 25 wherein said target polynucleotide is DNA.

37. The method of Claim 25 wherein said extending is carried out in the presence of nucleoside triphosphates.

38. The method of Claim 25 comprising adding to the product of step (c) a labeled oligonucleotide probe for each sequence being amplified capable of hybridizing to said sequence or a mutation thereof and determining whether said hybridization has occurred.

39. A method of producing multiple copies of a target sequence of a target polynucleotide, which comprises:

(a) providing in combination (1) a single stranded polynucleotide having a sequence that is said target sequence and that is flanked at each end by at least partially complementary first and second flanking sequences, (2) an oligonucleotide primer at least a 10 base portion of which at its 3'-end is hybridizable to that member of said first and second flanking sequences that is at the 3'-end of said single stranded polynucleotide, (3) nucleoside triphosphates, (4) a control polynucleotide, as a template to which said oligonucleotide primer hybridizes except for 1-10 nucleotides at the 3'-end of said oligonucleotide primer, and (5) a 3' to 5' exonuclease wherein said primer extends along said target sequence and said primer extends along said control polynucleotide only after said 1-10 nucleotides are degraded by said 3' to 5' exonuclease,

(b) incubating said combination under conditions for either wholly or partially sequentially or concomitantly (1) dissociating said single stranded polynucleotide from any complementary sequences, (2) hybridizing said oligonucleotide primer with the flanking sequence at the 3'-end of said single stranded polynucleotide and with said control polynucleotide, (3) extending said oligonucleotide primer along said

single stranded polynucleotide to provide a first extended oligonucleotide primer and degrading said oligonucleotide primer hybridized to said control polynucleotide and extending said degraded oligonucleotide along said control polynucleotide, (4) dissociating said first extended primer and said single stranded polynucleotide and dissociating said control polynucleotide and said extended degraded primer, (5) hybridizing said first extended oligonucleotide primer with said oligonucleotide primer and hybridizing said oligonucleotide primer and said control polynucleotide, (6) extending said oligonucleotide primer along said first extended oligonucleotide primer to provide a second extended oligonucleotide primer and degrading said oligonucleotide primer hybridized to said control polynucleotide and extending said oligonucleotide primer along said control polynucleotide to provide an extended degraded primer, (7) dissociating said second extended oligonucleotide primer from said first extended oligonucleotide primer and said extended degraded primer from said control polynucleotide, and (8) repeating steps (5)-(7) above, and

(c) detecting the presence of said extended degraded primer, the presence thereof indicating that said reagents and conditions for producing multiple copies of said target sequence of a target polynucleotide are functional.

40. The method of Claim 39 wherein said oligonucleotide primer is fully

complementary to that portion of said target sequence to which it hybridizes and is complementary to that portion of said control polynucleotide to which it hybridizes except for said 1 to 10 nucleotides at the 3'-end thereof.

41. The method of Claim 39 wherein a modified oligonucleotide primer is included in said combination wherein said modified oligonucleotide primer is substantially identical to said oligonucleotide primer except for a chemical modification at its 3'-end that prevents degradation, by said 3' to 5' exonuclease, of said 1 to 10 nucleotides.

42. The method of Claim 41 wherein said chemical modification is selected from the group consisting of phosphorothioates, ethyl phosphonates, carboxamides, sulfonamides, carbamates, acetals and ketals.

43. The method of Claim 41 wherein said chemical modification is a phosphorothioate.

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44. The method of Claim 39 wherein said oligonucleotide primer hybridizes to said control polynucleotide except for 3-5 nucleotides at the 3'-end thereof.

45. The method of Claim 39 wherein the presence of extended oligonucleotide primer is detected and related to the presence of said target polynucleotide.

46. The method of Claim 39 wherein said control polynucleotide is 50 to 5000 nucleotides in length.

47. The method of Claim 39 wherein said control polynucleotide is present in the reaction mixture at a concentration of from about 1pM to 100pM.

48. The method of Claim 39 wherein the repeating of steps (5)-(7) is achieved by repeated temperature cycling.

49. The method of Claim 48 wherein temperature cycling is repeated at least 3 times.

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50. The method of Claim 39 wherein said target polynucleotide is DNA.

51. The method of Claim 39 comprising adding to the product of step (c) a labeled oligonucleotide probe capable of hybridizing to said sequence or a mutation thereof and determining whether said hybridization has occurred.

52. The method of Claim 39 wherein said oligonucleotide primer is labeled with a reporter group.

53. A kit comprising in packaged combination:

(a) an oligonucleotide primer,

(b) a control polynucleotide having a sequence to which said oligonucleotide primer hybridizes except for 1-10 nucleotides at the 3'-end of said oligonucleotide primer,

(c) a modified oligonucleotide primer that is substantially identical to said oligonucleotide primer except for a chemical modification at its 3'-end that prevents degradation, by a 3' to 5' exonuclease, of said 1 to 10 nucleotides;

(d) nucleoside triphosphates, and

(e) a 3' to 5' exonuclease.

54. The kit of Claim 53 wherein said oligonucleotide primer is complementary to the portion of said control polynucleotide to which it hybridizes except for said 1 to 10 nucleotides at the 3'-end thereof.

55. The kit of Claim 53 wherein said chemical modification is selected from the group consisting of phosphorothioates, ethyl phosphonates, carboxamides, sulfonamides, carbamates, acetals and ketals.

56. The kit of Claim 53 wherein said chemical modification is a phosphorothioate.

57. The kit of Claim 53 wherein said oligonucleotide primer hybridizes to said control polynucleotide except for 3-5 nucleotides at the 3'-end thereof.

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